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The influence of food and temperature on enzymatic activities of the millipede *Glomeris hexasticha* (Diplopoda) *

BY

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Synopsis: Activities of three digestive enzymes, amylase, xylanase and cellulase of the millipede *Glomeris hexasticha* depended on its sex and were influenced by rearing temperature and diet composition. Xylan and starch were digested by examined animals very intensively but cellulose seems to be only a complementary source of energy.

Keywords: Millipede, sex, food preference, temperature, digestive enzymes, amylase, xylanase, cellulase.

INTRODUCTION

Millipedes play an important role in the decomposition processes of dead plant material. Food preference and consumption are influenced by many different factors (Bocock, 1964; Sakwa, 1974; David, 1987). During the passage of food through their digestive tract, millipedes are able to utilize, owing to their own enzymes and the presence of gut microflora, several components of ingested food. Many papers deal with digestive enzymes in the gut of these animals as well as with the function of microbial enzymes in the decomposition of gut content (Nielsen, 1962; Nunez & Crawford, 1976; Marcuzzi & Turchetto-Lafisca, 1976, 1977, 1978; Beck & Friebe, 1981; etc.).

* This article has been written *in memoriam* Claude Delamare Deboutteville, one of soil zoology founders.

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The aim of our study was to characterize the effect of food and temperature on the activity of digestive enzymes, amylase, xylanase and cellulase in the gut wall of both sexes of the millipede *Glomeris hexasticha* Brandt, 1833.

I. – METHODS

Millipedes were collected during April and May 1989 in a mixed oak forest near Netolice in South Bohemia, Czechoslovakia. Animals were reared in laboratory cultures on oak leaf litter (*Quercus robur* L.), decaying oak wood (fragments of branches) and on grass litter (*Calamagrostis arundinacea* L.) taken from the same locality. Plant material was dried under laboratory conditions and before each experiment it was moistened by distilled water on water content 64-78%. After removing the excess of water from the surface with filter paper, weighed aliquotes of the material were distributed to small Petri dishes (6 cm in diameter). Millipedes were kept for 1 week under conditions corresponding to subsequent variant of the experiment. Individual animals of both sexes were then placed to the Petri dishes with wet food into thermostats maintaining 7 and 15°C and a 12-hours photoperiod. Each variant had 7 or 8 repetitions. After 5 days the millipedes were taken for biochemical analysis.

Activities of enzymes alpha-amylase (EC 3.2.1.1), beta-xylanase (EC 3.2.1.8) and cellulase (exo- and endo-beta-1,4-glucanases) were determined in the gut wall of animals immediately after the termination of feeding experiments. Dissected gut was washed rapidly in distilled water until free from intestinal content. The excess of water was then removed from the preparation with a piece of cotton. The gut wall was weighed, homogenized under cooling in phosphate (Britton-Robinson) buffer of pH 6.0 and centrifuged for 10 minutes at 3,000 g. The pH of used phosphate buffer conformed with established pH-optima of amylase (5.5-7.0), xylanase (7.5) and cellulase (5.5) in the gut wall of examined animals and with data in the literature (Marcuzzi & Turchetto-Lafisca, 1978; Beck & Friebe, 1981). Optimum pH of enzymes was appointed with phosphate (Britton-Robinson) buffers of pH 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5. Supernatant of tissue homogenate was taken for biochemical analysis. Enzymatic activities were assayed by incubating 1 ml of enzyme solution with 50 mg (96 mg for cellulase) of specific chromolytic substratum (S-Tests, Lehnice, Czechoslovakia) at 37°C; 2-3 drops of toluene were added as a bacteriostatic. The tablet tests were developed in the Institute of Chemistry, Bratislava, Czechoslovakia (Zemek & Kuniak, 1983). The influence of reaction temperature on enzymatic activities was studied at 15, 25 and 37°C. Higher amylase, xylanase and cellulase activities were found at 37°C than at 15 and 25°C. Incubations were stopped after 2 hours (amylase), 3 hours (xylanase) and 29 hours (cellulase) by addition of a solution containing acetone and $NaCO_3$ in distilled water (10 g $NaCO_3$: 10 ml acetone : 900 ml H_2O). After centrifugation (5 minutes at 1,500-1,700 g is sufficient) the absorbance of a final colour was measured at 620 nm. The catalytic activity of enzymes was expressed in milligram of decomposed substratum per 1 hour and 1 g of fresh gut wall weigth. Each value was based on 6 parallel determinations.

Analysis of variance (ANOVA) was performed on all experimental results. Significant differences among factor levels were determined for alpha ≤ 0.05 (Student-Newman-Keuls Test). The Pearson's correlation coefficient and its significant level were calculated to verify the relationship between single enzymatic activities.

II. – RESULTS

Figures 1, 2 and 3 show amylase, xylanase and cellulase activities which were measured in the gut wall of both sexes of the millipede *Glomeris hexasticha* reared on three food substrata and at two temperatures. The influence of combinations

of investigated factors on the enzymatic activities of animals was very complicated, so that statistically significant differences in enzymatic activities were evident only for some variants of experiment.

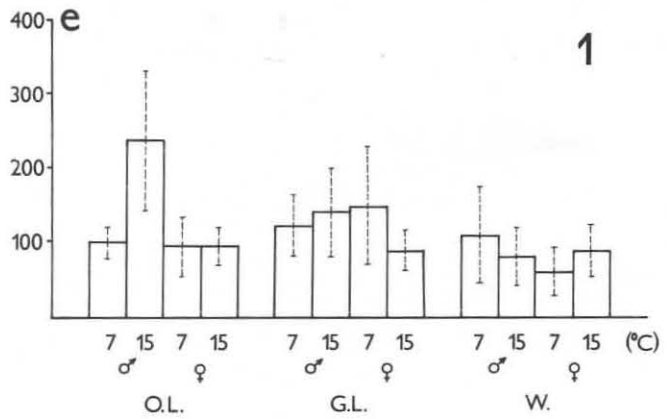

FIG. 1. – Amylase activities in the gut wall of *Glomeris hexasticha*. Abbreviations: O.L., oak leaf litter; G.L., grass litter; W., decaying oak wood; ♂, males; ♀, females; e, activity of enzyme in milligrams of decomposed substratum per 1 hour and 1 g of fresh gut wall weight; vertical lines, 1 SD.

Amylase activity (*Fig.* 1) was higher in the gut wall of males reared on oak leaf litter at 15°C than at 7°C. Males had higher amylase activity than females reared on oak leaf litter at 15°C.

Higher xylanase activities (*Fig.* 2) were measured in males than in females of *Glomeris hexasticha* reared on decaying oak wood at both temperatures.

Cellulase activity (*Fig.* 3) was higher in males on grass litter at 15°C than at 7°C. Males had higher cellulase activities than females reared on grass litter and decaying oak wood at 15°C.

For that reason analysis of variance was calculated for single examined factors, food, temperature, and at the same time for differences in enzymatic activities between males and females of the millipedes.

Amylase activity (Tab. I) was on the average higher in the males than in the females reared under experimental conditions. No significant influence of the rearing temperature on the amylase activity of millipedes was observed. Higher enzymatic activity was found on the average in the gut wall of animals reared on oak leaf litter and grass litter than on decaying oak wood.

Xylanase activity (Tab. I) was on the average higher in the gut wall of the males than that in the females. A statistically significant effect of the rearing temperature on average xylanase activity of animals was not detected. Higher xylanase activity was found on the average in millipedes reared on oak leaf litter than on grass litter and decaying oak wood.

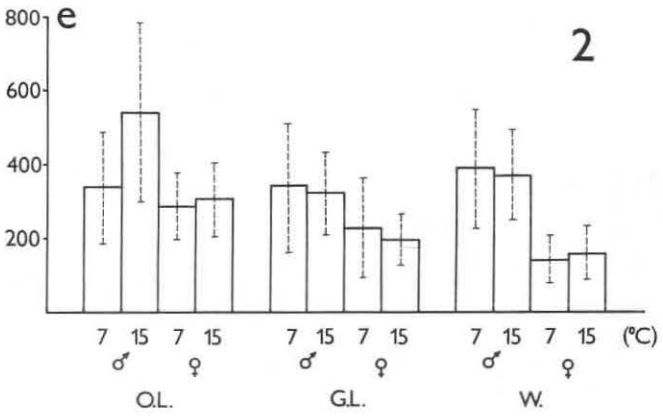

Fig. 2. – Xylanase activities in the gut wall of *Glomeris hexasticha*. Abbreviations: see Figure 1.

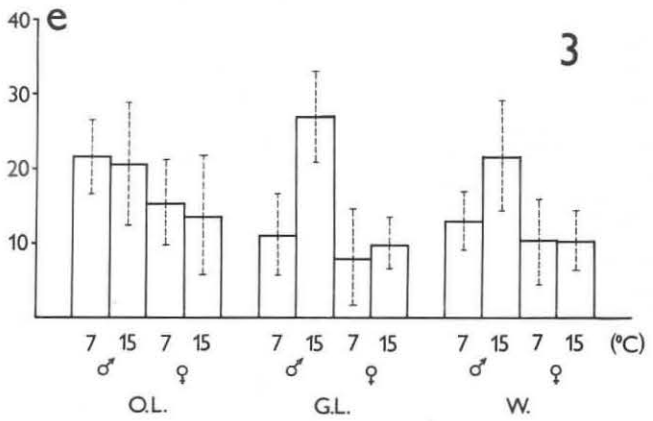

Fig. 3. – Cellulase activities in the gut wall of *Glomeris hexasticha*. Abbreviations: see Figure 1.

Cellulase activity (Tab. I) was on the average higher in the gut wall of the ıales than that in the females. The millipedes had on the average higher cellulase ctivity in cultures at 15°C than at 7°C. The individuals reared on oak leaf litter ad on the average higher cellulase activity than those on grass litter and decaying ak wood.

Results of the present study showed generally high xylanase and amylase ctivities in the gut wall of millipedes. Very low cellulase activities were determined all cases.

TAB. I. – **Differences in enzymatic activities of millipedes *Glomeris hexasticha* influenced by investigated factors.** Abbreviations: E, average activity of enzyme in milligrams of decomposed substratum per 1 hour and 1 g of fresh gut wall weight; Alpha, significant level; *n*, number of repetitions; see Figure 1.

		E	Alpha	*n*
Amylase				
Sex	♂ ♀	133.36 98.14	0.0083 **	36
Food	O.L. G.L. W.	134.65 126.65 85.94	0.0066 **	24
Temperature	7°C 15°C	107.48 124.02	0.2047	36
Xylanase				
Sex	♂ ♀	387.63 224.12	0'0000 ***	36
Food	O.L. G.L. W.	371.22 276.87 265.53	0.0164 *	24
Temperature	7°C 15°C	291.75 320.00	0.3689	36
Cellulase				
Sex	♂ ♀	19.26 11.27	0.0000 ***	36
Food	O.L. G.L. W.	17.96 14.09 13.75	0.0290 *	24
Temperature	7°C 15°C	13.24 17.29	0.0051 **	36

High positive correlation ($r=0.575$, $N=72$, alpha $\leqq 0.01$) was found between activities of xylanase and cellulase measured in the gut of investigated millipedes. Low positive correlation ($r=0.305$, $N=72$, alpha $\leqq 0.01$) was detected between activities of amylase and xylanase while correlation between activities of amylase and cellulase was non-significant.

III. – DISCUSSION

Results of many authors (NIELSEN, 1962, 1963; MARCUZZI & TURCHETTO-LAFISCA, 1976, 1978; BECK & FRIEBE, 1981) showed a presence of oligosaccharases and polysaccharases in the gut of millipedes. In those experiments the determinations of enzymatic activities were carried out in whole gut with gut content and so investigated enzymes could be produced by tissues of the gut wall or by microorganisms in the gut content of animals.

Some authors presumed production of animal's own enzymes because they measured enzymatic activities only in the gut wall of millipedes. STRIGANOVA (1971) published the occurence of cellulase activity in the gut wall of *Pachyiulus foetidissimus* and a very intensive cellulose degradation in the gut content of *Pachyiulus foetidissimus*, *Cylindroiulus ruber* and *Julus colchicus*. NUNEZ & CRAWFORD (1976) detected high oligosaccharase and also amylase, xylanase, pectinase, lipase and C_x-cellulase activities in the midgut tissue of *Orthoporus ornatus*.

Other authors reported the absence of animal's own cellulase in the gut of millipedes and published the dependence of these animals on symbiotic microflora (NIELSEN, 1962; MARCUZZI & TURCHETTO-LAFISCA, 1976). TAYLOR (1982) found aerobic bacteria in the gut of desert millipedes *Orthoporus ornatus* and *Comanchelus* sp. She assumed that the millipede-bacteria association is mutualy beneficial and makes avaiable an otherwise mostly unutilizable substrate (cellulose, xylan) to millipedes. She also measured the highest cellulolytic activity in the midgut of investigated animals. But BIGNELL (1989) published that microorganisms colonizing leaf litter were a valuable nutritional resource to millipedes and were assimilated with high efficiency. Scanning electron microscopy of the food, gut contents and faeces of *Glomeris marginata* showed that colonizers were stripped from leaf litter in ingestion, but also indicated that recolonization of faecal pellets by bacteria occurred during or immediately after transit of the hindgut.

Transmission electron microscopy studies of food, gut content and faecal pellets of the millipede *Glomeris hexasticha* confirmed this alteration of microbial communities in the digestive tract owing to bacterial and fungal lysis in the midgut and subsequent recolonization by microbial symbionts in the hindgut (TAJOVSKÝ *et al.*, in press).

Interesting conclusions were reported by MONROZIER & ROBIN (1988). They investigated changes in the composition of hornbeam (*Carpinus betulus*) litter resulting from ingestion and digestion by the millipede *Glomeris marginata*. These changes were studied by elemental analysis (C, H, N) and pyrolysis of fresh litter and corresponding faecal pellets. Comparison of pyrograms and C/H ratios showed that cellulose survived passage through the gut of these animals.

Results of the present study documented the presence of active digestive enzymes, amylase, xylanase and cellulase in the gut wall of both sexes of millipede *Glomeris hexasticha*. Enzymatic activities were determined in washed gut wall of millipedes with toluene as a bacteriostatic and so these enzymes were largely of animal's own origin. The view that millipedes could secrete own enzymes in their gut wall was also supported on differences in cellulase pH-optima of the gut wall and the gut content of 4 animals. The gut wall of millipedes contained cellulase which had maximal axtivity in acid area. Three pH-optima (in acid, neutral and alkaline areas) were measured in gut contents. Similar results were also found in earthworms (URBÁŠEK, 1990).

Amylase and xylanase catalytic activities were much higher than those of cellulase. It is possible to suppose that the millipedes digested preferably starch and xylan in their food. On the contrary cellulose seemed to be only a complementary source of energy.

The relationship between activities of xylanase and cellulase was examined by statistical analysis which revealed a high positive correlation. A low positive correlation was found between amylase and xylanase activities while the relationship between amylase and cellulase activities was statistically non-significant. It

seems that the digestion of xylan and cellulose proceeds in the gut of millipedes parallelly. This fact is probably related to the simultaneous occurrence of structural polysaccharides xylan and cellulose in plant materials.

The males of *Glomeris hexasticha* had usually higher values of all investigated enzymes than the females. The lower enzymatic activities in the females could be due to their sexual activity (vitellogenesis in ovaries) in the investigated period (April-May). During this period the females appear to use the reserves accumulated in their fat body.

The present results showed higher digestion of starch, xylan and cellulose by millipedes reared on oak leaf litter than on grass litter and decaying oak wood. The lower utilization of some food by these animals could by explained by higher contents of lignin, neutral oils, waxes and esters. In the experiments of NEUHAUSER & HARTENSTEIN (1978), millipedes were unable to degrade lignin. MARCUZZI & TURCHETTO-LAFISCA (1977) reported very low lipase activities of *Glomeris euganeorum*, *G. undulata* and *G. conspersa*.

The influence of rearing temperature on the enzymatic activities of investigated animals was expressed only by higher cellulase activity in the gut of millipedes at 15°C than at 7°C. The effect of temperature was evidently modified by the interactions of this factor with the influence of food on the enzymatic activities of both sexes of millipedes. Investigated temperature extent was probably too close for the evocation of visible response in changes of enzymatic activities of animals.

It is necessary to emphasize that the presented data described the metabolic activity of millipedes collected during spring period. KHOTKO & TARASEVICH (1987) reported that millipedes changed their ability of the assimilation of same leaf litter during year.

Moreover results of the present study assess activities of digestive enzymes measured in the gut wall of millipedes. Decomposition of ingested food goes off in the gut cavity of animals where metabolic activity of digestive enzymes secreted by millipedes is probably completed by microbial enzymatic activities.

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SUMMARY

The influence of food and rearing temperature on the activities of digestive enzymes amylase (EC 3.2.1.1), xylanase (EC 3.2.1.8) and cellulase (exo- and endo-beta-1,4-glucanases) were measured in the gut wall of both sexes of the millipede *Glomeris hexasticha* during spring period. Specific chromolytic substrata (S-Tests, Lehnice, Czechoslovakia) were used for the determination of enzymatic activities and investigated enzymes were largely of animal's own origin.

The males of *Glomeris hexasticha* had higher catalytic activities of their digestive enzymes than the females. Higher cellulolytic activity was found in the gut wall of millipedes at 15°C than at 7°C. Examined animals digested starch, xylan and cellulose more intensively

in oak leaf litter than in grass litter and decaying oak wood. Present results also indicated prior utilization of xylan and starch in food by millipedes while cellulose was probably only a complementary source of energy. The utilizations of xylan and cellulose seem to go in parallel ways.

Résumé

Influence de la nourriture et de la température sur l'activité enzymatique du diplopode *Glomeris hexasticha*

L'influence de la nourriture et de la température sur l'activité des enzymes digestives amylase (EC 3.2.1.1), xylanase (EC 3.2.1.8) et cellulase (exo- et endo-beta-1,4-glucanases) a été suivie dans la paroi intestinale du diplopode *Glomeris hexasticha* au cours du printemps. Des substrats spécifiques ont été utilisés pour la détermination de l'activité enzymatique.

Les mâles de cette espèce ont une activité catalytique de leurs enzymes digestives plus importante que celle des femelles. Une activité cellulolytique dans la paroi intestinale plus élevée a été constatée dans les élevages menés à 15°C par rapport à ceux menés à 7°C. Les animaux étudiés ont digéré l'amidon, le xylane et la cellulose d'une façon plus active dans les feuilles de chêne que dans la litière herbacée et les rameaux de chêne. Les résultats mettent aussi en évidence une utilisation préférentielle du xylane et de l'amidon dans le matériel consommé alors que la cellulose n'est probablement qu'une source complémentaire d'énergie. Les utilisations du xylane et de la cellulose ont lieu à peu près simultanément.

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